

# PHOTOMODULATION METHODS AND DEVICES FOR REGULATING CELL PROLIFERATION AND GENE EXPRESSION

## Background of the Invention

### 1. Field of the Invention

This invention relates to method and devices for the regulation of cell proliferation and gene expression. In particular, the invention relates to the inhibition of photoaging of the skin.

### 2. Description of the Background

Chronological aging brings about a group of changes in the appearance of human and mammalian as well as changes in the structure and function of the skin. All living cells, tissues and organs also undergo changes associated with chronological aging. Since the human skin is an organ that is highly visible, the changes associated with chronological aging are readily apparent and visible. These changes are reflections of the underlying structural and functional changes.

The phenotype associated with chronological aging of the skin is an outward reflection and expression of the genotypic changes, which occur within the cells of the skin. The most widely appreciated form of skin aging is that which is produced by over exposure and repeated chronic exposure to sunlight and is generally termed photoaging. More specifically certain portions of the ultraviolet A (UVA) and ultraviolet B (UVB) and have been determined to be the principal causative factors of what are associated with photoaging.

For many years it was thought that photoaging occurred through a different mechanism of action or and was somehow different than chronological aging. However, more recently it appears that photoaging and chronological aging may share similar, if not identical pathways.

Solar radiation is composed of ultraviolet (UV), visible and infrared, light. Current conventions divide UV radiation into UVA (320-400 nm), UVB (290-320 nm) and UVC (<290 nm). UVC radiation is blocked by ozone in the stratosphere and does not reach the earth's surface, but can be generated by germicidal lamps and other machinery. UVA and UVB sunlight do reach the earth and are believed to be the principal agents of photoaging. UVA radiation is further subdivided into UVA 1 and UVA 2. While UVB has been believed to be the primary agent for photoaging, it is now appreciated that certain wavelength ranges within the UVA rays

also contribute to changes associated with photoaging.

UVA and UVB light exposure to human skin triggers a series of molecular events including the induction of reactive oxygen species (ROS) in the skin. Through a series of cell signaling events collagen production is down regulated and various enzymes known to degrade structural proteins in the skin up-regulated. The net result of this is a decrease in collagen and the production of wound. The skin's reaction to UVA or UVB (or combined) wounding is to repair the wound through the skins wound healing mechanism. Typically these wound repair mechanisms are imperfect which is considered by many to be a solar scar. After many years of the UVA or UVB wounding of the skin, chronic solar scarring develops which manifests itself in the visible phenotypic changes termed photoaging, which might also be considered the visible outward evidence of solar scars.

Photoaging of the skin may occur through acute injury at higher levels, such as what one associates with sunburn. This triggers an inflammatory process in the skin and the associated cellular mechanisms. There is also a more chronic low-level type of injury that does not produce a sunburn reaction, but which produces the changes of chronic photoaging. Other processes, which are known to decrease collagen production and increase collagen-dissolving enzymes, such as tobacco smoking, also are associated with changes that visibly appear, similar to the photoaging from UVA/UVB light. This can be seen strikingly in photographs of identical twins wherein only one twin smoked tobacco for many years.

UVB radiation in sufficient doses produces reddening or sun burning of the skin. The threshold level is typically described as minimal erythema dose (MED), typically produced by 290-300 nm UVB wavelengths. As the wavelengths increase they become much less likely to produce the redness and burning reactions and indeed wavelengths of 320 nm are about 100 times as powerful as wavelengths of 340 nm approximately 100 times less powerful than the 290-300 nm range of producing erythema and sunburns. The total UVB exposure is more related to the appearance of photoaging and sunburns are more likely to trigger malignant changes in the skin such as malignant melanoma. In contrast, UVA radiation can produce redness, but also produced tanning and these are the wavelengths typically used for the so-called tanning beds.

UVA radiation is a longer wavelength and is proportionately greater in the early morning and late afternoon and the UVB rays, which are typically most predominant and intense at the midday summer sun time exposure period, UVA radiation may also penetrate certain sun blocks and certain sunscreens and also window glass on automobiles, thus accounting for the frequently observed greater wrinkling, brown pigmentation and redness and overall aged appearance on the left side of the face than the right in patients who occupationally or recreationally spend considerable time driving a left hand drive motor vehicle.

In sunny countries with fair complexioned populations, such as Australia, where right hand drive motor vehicles are used, these changes are seen typically seen on the right side of the face. The patterns of photoaging are determined by which areas of the body are anatomically are more chronically exposed to sunlight. Thus, the face, neck, back of hands, upper chest, lower arms, lower legs and depending on hair styling and density, ears and balding areas manifest the greatest photoaging changes.

The chronological changes and photoaging changes typically are manifest by fine lines and wrinkling of the skin. A coarser, crepey texture to the skin, skin laxity and skin sagging, uneven pigmentation, brown splotchy pigment, loss of skin tone, texture and radiancy, bruising and sallowness. The skin is composed of several layers, the outermost layer is called the stratamocornium (SC), next layer is the epidermis (EPI), and underneath the epidermis lies the dermis (DER). The outer SC serves primarily a barrier function to protect the skin from environmental exposure and also to help minimize water loss from the skin. The epidermis serves many important and diverse roles as does the dermis. The dermis contains the principal structural proteins of the skin. These proteins are collagen, elastin and ground substance. They are manufactured by the fibroblast cells within the dermis. Fibroblast cells control the activity to produce these proteins as regulated by a complex and relatively well defined series of cell receptors and cell signaling mechanisms.

The proliferation of these cells is also an important activity. For example, the dermis also contains blood vessels, nerve fibers, oil and sweat glands, hair follicles and many other important components. There is a remarkably complex inner communication through cell signaling in the

cells of the skin. Fibroblasts produced what are termed pro-collagen fibers, which are then insymmetrically assembled into collagen fibers, and form bundles within the dermis. Other molecules, such as decorin affect the function of the collagen. There are various sub-types of collagen fibers such as Collagen I, III, etc., within the body. Collagen I comprises approximately 85% of the skin and Collagen III approximately 10%. However, in photoaged skin the amount of Collagen I decreases so the ratio of Collagen III/I is altered.

There are also a variety of enzymes termed matrix metalloproteinases (MMP) which play important roles in aging skin. Fibroblasts also have important functions in wound healing with the removal of damaged structural ECM and the repair and production of (ECM). The Collagen I is degraded principally by MMP 1 (collagenase). There are a variety of MMP enzymes, which degrade one or more of the structural proteins in the skin. While these degrading MMP enzymes serve an important role in removing damaged skin (for example, in wound healing), their activation and synthesis in increased quantities in normal skin helps contribute to the changes seen in both chronological and photoaging. Likewise, if the production of Collagen I is decreased or diminished this results in changes which are associated with chronologically or photoaged skin. Aging or senescent fibroblasts may exhibit decreased synthesis of Collagen I and increased synthesis of MMP 1. Similar changes are seen with UVA/UVB exposure. Other environmental agents may produce similar changes.

Certain drugs, therapies, chemicals, active agents have been demonstrated to reversing the appearance of or phenotype of a chronologically aging or photoaging skin. Some topically applied agents serve as a physical or optical barrier either by reflection or absorption of ultraviolet light thus protecting the skin. There are also enzymes that have been shown actually repair the DNA dimers which are produced from UV damage. Other topically applied or oral or systemically agents have been shown to improve the appearance of the skin. One of the classic and well-known agents is a topical Vitamin A derivatives termed Retinoids. Numerous studies have demonstrated the ability to improve the appearance or phenotype of photoaged skin with the use of all-trans retinoic acid (RA). Many of the pathways involve the mechanism of action of RA and also Retinol (RO). Much of the mechanism of action in the cell signaling

pathways through which RA appears to produce anti-aging effects.

One of the goals of some current anti-aging therapies is to increase production of collagen in the ECM and the dermis of the skin. Some believe collagen I is the more desirable form of collagen to increase. There is some support for this since photoaged skin has less desirable visco elastic properties and this is thought in part to be due to the increased proportion of collagen III to collagen I. Other anti-aging approaches indicates that reducing the activity or production of the degrading enzymes in the ECM will similarly produce an anti-aging effect in the appearance of the skin. Doing a combination of both is even more beneficial. An analogy one might make is the production of new collagen I and that of freshly newly fallen snow. The amount of accumulation of the fresh snowfall is dependent both on the amount of snow that is fallen as well as the amount of the freshly fallen snow which then melts. Thus one could envision an anti-aging therapy which stimulated new collagen production (newly fallen snow). When a piece of black asphalt in a parking lot abuts a piece of warmer black asphalt adjoins a colder piece of concrete or frozen ground, while the amount of new snowfall is equal in both areas, the amount of accumulated snow is less was melted by the asphalt. If an anti-aging therapy stimulates collagen I production, but does not diminish MMP 1 activity, the net increase in collagen I will be smaller than if the MMP 1 activity is also decreased.

Historically there have been many approaches to restoring a youthful appearance to human skin for achieving anti-aging or age reversal therapies. Most methods utilize some form of triggering the body's own wound healing mechanism. The more destructive and traumatic methods use chemicals to peel off the stratum corneum epidermis and often a portion of the dermis or they mechanically abraded by sand papering or dermabrating or more recently high-energy thermal lasers have been used to vaporize or coagulate the skin. These methods have a prolonged and painful wounding period and require wound care and patients typically must limit their daily social and business activities during the wound-healing phase. Subsequently the skin undergoes of months or years an on going wound healing and wound remodeling process whereby damage is repaired and new structural proteins in skin are generated. These treatments typically amount to trying to produce a controlled entry to the skin and proving the wound care

environment that minimizes the risk of scarring. These methods are notoriously known for producing many problems and sometimes even disfiguring scarring or catastrophic pigment changes in the skin. However, properly performed and with good wound care, many people achieved significant and sometimes dramatic anti-aging effects. Other gentler methods have become more popular in recent years which involve the classic plastic surgery lifting procedures and newer procedures termed non-ablative where the outer stratum corneum and epidermis are not removed or blated from the skin, but are by various means and methods protected and left intact. Non-ablative methods have typically been thermal in nature and through various means of laser light, intense pulsed light, radio frequency or microwave energy delivery then produced a thermal injury to the dermis. The theory behind these therapies is that this injury will result in a net increase in the desirable structural proteins, while not triggering, worsening, scarring or other complications. Results are occasionally traumatic but have been extremely variable with this therapy. The variability in individuals wound healing repair mechanism and the overall health of their body and skin and many other factors contribute to this variability.

There are various topical agents that have been developed for anti-aging purposes such as Retinoic acid, topical Vitamin C, topical Vitamin E and other antioxidant and other anti-wrinkle creams and lotions. Many of these are well defined.

There is a need to improve the appearance of chronologically aged, photoaged, or environmentally damaged skin, but without producing the risk, complications, recovery time, pain, discomfort, wound care or other side effects traditionally associated with surgical, chemical, electromagnetic radiation and other types of therapies.

### **Summary of the Invention**

As embodied and broadly described herein, the present invention is directed to method and devices for improving the appearance of photoaged or damaged skin. Methods and devices involve the regulation of cell proliferation and gene expression of skin and other cells.

One embodiment of the invention is directed to methods for both inhibiting, as well as reversing the appearance of photoaging (beauty maintenance) or chronological or environmentally damaged induced aging of human skin by application of photomodulation by,

for example LED or other electromagnetic radiation treatment. Preferably, the invention is directed to the regulation of cell proliferation of cells of the skin, and/or the regulation of gene expression in such cells.

Another embodiment of the invention is directed to the various genotypes that characterize different phenotypes of aging skin and also a database comprising a collection or library of such phenotypes. The data base may comprise a plurality of genotypes identified from a variety of different individuals with the same disorder, or a variety of individuals with different disorders.

Another embodiment of the invention is directed to photomodulation by light or electromagnetic radiation so as to effect cell proliferation and/or gene expression. Examples of different types of electromagnetic radiation include ultrasound, radiowaves, micro rays, magnetic fields, any electrical stimulation that produces changes in the genotype or phenotype of aging skin, and combinations thereof.

Other embodiments and advantages of the invention are set forth in part in the description, which follows, and in part, may be obvious from this description, or may be learned from the practice of the invention.

### **Description of the Invention**

As embodied and broadly described herein, the present invention is directed to method and devices for the regulation of cell proliferation and gene expression and, in particular, the inhibition of photoaging of the skin.

Photoaging of the skin occurs through many mechanisms, including, for example, environmental factors such as tobacco smoke, exposure to the sun, and poor health, to name a few. These events can triggers an inflammatory process in the skin and the associated cellular mechanisms. There is also a more chronic low-level type of injury that does not produce a sunburn reaction, but which produces the changes of chronic photoaging. Chronological aging of the skin and photoaging and other environmentally induced changes share many or in some cases, all of the same pathways as UV induced photoaging of the skin. These pathways involve up and/or down regulation of cell proliferation and also alterations in the level of expression of

many different types of genes.

It was surprisingly discovered that, this combination of regulation of cell proliferation and regulation of gene expression, is responsible for photoaging of the skin and other cells and tissues, and thus, photoaging could be reversed or at least ameliorated by affecting these same processes. Accordingly, one embodiment of the invention is directed to identifying and correlating the phenotypic and genotypic expression characteristics of photoaging and other skin and cell-associated disorders. Once identified, correlated maps can be compiled and collected into a data base to allow for the rapid and efficient identification of similar disorders and conditions for the purpose of tailoring appropriate treatment. Further, once identified, treatment and appropriate intervention and prevention methods can be used to halt or even reverse the appearance and genotypic characteristics of photoaging. Thus, the invention is not directed to artificially hiding or covering up aspects associated with aging, but actually reversing the processes and mechanisms associated with aging-related phenomena.

A further embodiment of the invention is directed to applying these same mechanisms and tools to other cells such as stem cells (completely undifferentiated cells) and progenitor cells (partially differentiated cells). By altering the cell cycle, cell proliferation, and/or gene expression characteristics of these cells along defined parameters, it is possible to determine differentiation pathways and to create or recreate cells, tissues and other cell structures for disease therapy and prevention, and wound healing.

Methods to modulate cell proliferation and gene expression include exposure to electromagnetic radiation in an amount or dose that is sufficient to stimulate the desired effect (e.g. see U.S. Patent Nos. 6,398,753, 5,837,224, and 6,130,254; and U.S. Patent Application Nos. 2002/0028185, 2001/0053347, 2003/0004556, 2003/0004499, and 2002/0123746, all of which are specifically and entirely incorporated by reference). For example, exposure of skin to LED can stimulate or inhibit the expression of various gene products. These same methods can be used to cause stimulation or inhibition of cell proliferation and cell cycle modulation in these cell populations. Further, photomodulation can be used in combination with certain oral agents (for systemic affects) or topical agents (for localized affects) (e.g. vitamin A, retin A, retinol), for a



desired effect unachievable with either stimulant used individually.

The types of cells that can be affected include, but are not limited to skin cells (reversal of photoaging), nerve cells (disease prevention and treatment), stem cells (tissue reconstruction), cells of hair follicles (hair growth or inhibition), cells of the immune system including cells intimately involved with the process of inflammation (due to disease, infection, or congenital disorder), wound repair, and combinations thereof. Modulation can be achieved by exposing cells to electromagnetic radiation (e.g. photomodulation) such as, preferably, visible light, (e.g. purple, blue, green, yellow, orange, red), infrared radiation, ultraviolet light (UVA, UVB, UVA1, UVA2, or combinations thereof), or combinations of any. Preferred exposure strengths and exposure times are as set forth in the attachments hereto, but may include pulsed exposures, continuous and periodic exposures.

#### **Modulation of Gene Expression**

Ultraviolet light injury triggers reactive oxygen species and a series of cell signaling events called kinase cascades. One of the final common pathway in the up and down regulation of fibroblast activity is through AP-1 which up regulates and increases the production of various MMP's including MMP 1 (collagenase 1 or interstitial collagenase synthesis), MMP 9 (gelatinases B) and MMP 3 (stromelysins 1). The production of these MMP enzymes results in the breakdown of collagen, elastin and ECM in the dermis of the skin. Simultaneously the actual production of collagen I and other structural proteins may be diminished or down regulated thus further accelerating the process.

The aging of living cells, tissues and organs may be related to free radical exposure and oxidative stress. To apply this model to aging skin, chronological aging results from a decrease antioxidant defense mechanisms while UV photoaging and other environmental stresses can be thought of as increasing oxidative stress. The net result of decreased antioxidant defense or increased oxidative stress is increase production of (ROS) or free radicals.

#### **Modulation of Gene Activity**

Increased ROS production in the skin stimulates cell signaling or signals transduction pathways, which produce altered gene activity. Damage to structural proteins (e.g. damage,

disruption and fragmentation of collagen caused by UV light) alters proteins, structure and function which in turn changes cell signaling and may alter gene activity. Another possible outcome of increased ROS production is the production of DNA mutations, which then alters gene structure and thus may alter the normal structure and function of cells. Much of the variation in the human state, as far as disease and response to environmental insults may be mediated by relatively small differences in the genetic make-up from one individual to the next. Single nucleotide polymorphisms (SNPs) are currently being very actively investigated as a means of identifying and potentially predicting the differences in biological responses of humans and other animals. For example, characterization of SNPs may allow prediction of whether a patient is more or less likely to develop a specific disease or tumor and thus take known preventative measures. Another possible application is the use of SNPs to screen individuals before placing them on a prescription drug to identify those individuals who might be more likely to develop serious side effects and thus avoid the use of that drug. Another potential novel use of SNPs is to identify the haplotype or patterns of SNPs, which are associated with, for example, chronological aging of the skin. Some individuals and families have reduced risk of skin cancers or simply look younger than their peers of the same age group and like backgrounds. A profile of SNPs can be developed that characterizes common factors associated with the phenotypic changes of aging skin (defined the SNP genotypic pattern that puts an individual at a greater risk of accelerate aging from increased oxidative stress from environmental agents). This allows for a treatment plan, which would have greater anti-aging benefits.

TGF- $\beta$  is a major cytokine for cell signaling and inhibits the growth of epidermal keratinocytes and stimulates the growth of dermal fibroblasts. It also induces synthesis and secretion of the major collagen elastin and inhibits the expression of MMP 1 and MMP 3. There are multiple TGF- $\beta$ 's, TGF- $\beta$  1, TGF- $\beta$  2, TGF- $\beta$  3, many of which are down regulated in aging skin cells. TGF- $\beta$  is also activity altered in aging skin by binding with Decerin and when this combines with collagen affects the tensile strength of skin as well as controlling the rate of collagen fiber formation. c-Jun mRNA is doubled in activity and age human skin compared to young skin but c-fos was unchanged. MMP 2 is not regulated through AP 1. ERK activity is

reduced in aging skin, but JNK activity is increased 3-4 times in aging skin. Environmental insults-damage can vary anatomically over a person's body. These methods allow for rejuvenating human skin including the steps of simultaneously preventing collagen degradation while also stimulating the formation of new collagen in aging human skin.

Increased MMP's result in reduced levels of ERK, cyclin D2 and type I and III pro collagen. This is part of the core genotype, phenotype stimulating a number of keratinocytes as well as decreasing c-gen activity and increasing ERK activity.

A system of sunscreens, topical oil and antioxidants, topical oil and photomodulated ECM stimulation and MMP and MMP inhibition and various combinations and mixtures of the above. Inhibiting c-gen formation also inhibits formation AP-1 and thus diminishes MMP's, inducing the proliferation of keratinocytes and fibroblasts.

#### **Modulation of Mitochondrial Activity**

Mitochondria and ATP production mechanisms (e.g. cytochrome expression) can be modulated by electromagnetic radiation. LED light activates cell surface receptors via redox mediated in activation or a receptor type protein tyrosine phosphatase (RTPT). SAP (stress activated pathways) verses mitogen activated pathways compare and contrast SAP increase MMP and decreases pro collagen 1 and 2 if c-gen goes up. Primarily has to do with the ECM production whereas the MAP pathways activate IRK induced cyclins and promote cell growth so that PSAT's tend to increase or decrease protein production whereas the MAPS increase or decrease cell growth. Ras/MAP/AP-1 pathway plays a key role in response to wounding. FGFR1 contains sites in the promoter region and IL1 antagonist promoter. Antioxidant compounds also have anti-erythema sunscreen effect although they may not inhibit the increased MMP after UV exposure, lycopene is one of these. LED photomodulation can also be used to diminish sunburn activity and MMP levels were maxed about 24 hours later. Use a solar simulator to cause a one MAD minimal erythema dose on the arm in two places on volunteers and treat one a couple times a day with the GW device and to reduce redness with the chromometer. Biopsy will show what happens when you treat them with GW after UV. Inhibiting cytochrome P-450 breakdown of retinoids increases retinoid strength concentration.

While not wishing to be constrained to a particular theory of operation, the invention includes the surprising discovery that multiple receptor-mediated pathways may be photomodulated in human or mammalian skin that lead to an expression of the genotype associated with a younger or more youthful or less aged skin both in appearance and structurally and functionally.

Reference to infrared-a radiation induced MMP 1. Infrared is capable of producing MMP 1 by way of up regulation or activation of MAPK signaling pathway that is the activation of ERK 1/2 that the promoter region of the MMP 1 gene was activated by IRA without the production of heat, but that TIMP 1 was not increased. MMP-8 or elastinase is increased with inflammatory reaction, which also involves AP 1. And when NF-KB is increased it activates more of IL-1 and TNFa that discontinues the presence of continued inflammation.

Fibroblasts sensor matrix surround them and when in contact with a matrix they tend to be less active produce little collagen, but when the presence of collagen breaks down products such as gelatin, they tend to produce more collagen if the inflammation persists. The collagen not only proliferates, but produces less scarring.

Topical compounds that inhibit cytokines are indirect MMP inhibitors because if they block the pathway the signals MMP the essentially block this. The same is true for MMP regulation. Regarding nutraceuticals, Vitamin C can be topically applied to assemble stable collagen molecules. Collagen I and collagen III can be stimulated by topical of Vitamin C, whereas elastin, Fibrilin 1/2 are not affected nor is MMP 1, 2, and 9 affected. TIMP was increased, TIMP 2 was unchanged.

#### **Modulation for Wound Healing and Therapy**

Proteolytic degradation of ECM is an essential feature of repair and remodeling during continuous wound healing. Wound repair consists of narcotic or damaged tissue, cell and/or tissue migration, angiogenesis, remodeling of newly synthesized ECM, and cell growth factor regulations. During wound repair MMP 1 and MMP 3 increase as well as MMP 2 and 9. MMP 13, in particular, for chronic wounds, but also acute. TIMP is also altered. MMP 1, 3, 9 are increased with UVB; increased elastin and fibrilian verscian; result in the formation of non

functional elastin fibers and reduce skin elasticity and aging or photoaged skin. Collagen I is reduced, and UVA shows increased expression of MMP 1, 2, 3.

Disease states-systemic scleroderma skin fibroblasts produced less MMP 1 and MMP 3 and more TIMP 1 compared to normal. Skin cancers BCC produce more MMP 1, 2, 9 and 11. More signs of photoaging, bruising, skin hypopigmented areas, fibrosis. Methods and inventions for preventing the photoaging or chronological or environmental aging of unaged skin include retinoids that retard the effects of photoaging topical antioxidants to reduce presence of ROS in the skin. Environmental stresses include oxidants, heat, UV light. Thus, LED phototherapy is both an ECM protein/collagen stimulator, and an MMP inhibitor. Dose dependent UVB induction of AP 1 and NF-KB, these induced MMP 2 and MMP 9. The formation of collagen bundles is responsible for the strength, resiliency and elasticity of the skin.

The following examples illustrate embodiments of the invention, but should not be viewed as limiting the scope of the invention.

#### **Examples**

Attached hereto are PowerPoint presentations of the various embodiments of the invention, and lists of gene products which can be regulated by methods of the invention.

Other embodiments and uses of the invention will be apparent to those skilled in the art from consideration of the specification and practice of the invention disclosed herein. All references cited herein, including all publications, U.S. and foreign patents and patent applications, are specifically and entirely incorporated by reference. It is intended that the specification and examples be considered exemplary only with the true scope and spirit of the invention indicated by the following claims.